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Response of poly-phosphate accumulating organisms to free nitrous acid inhibition under anoxic and aerobic conditions

Yan Zhou [a,*], Lily Ganda [a], Melvin Lim [b], Zhiguo Yuan [c], Wun Jern Ng [a]

[a] Advanced Environmental Biotechnology Centre (AEBC), Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological University, SBS-B2N-01, 60 Nanyang Avenue 639798, Singapore
[b] Keppel Environmental Technology Centre Pte Ltd, 61 Old Toh Tuck Road 597656, Singapore
[c] Advanced Water Management Centre (AWMC), The University of Queensland, Australia

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ABSTRACT

The response of free nitrous acid (FNA)-adapted poly-phosphate accumulating organisms (PAOs) to FNA inhibition under aerobic and anoxic conditions was studied. Anoxic P-uptake was 1–6 times more sensitive to the inhibition compared to aerobic P-uptake. The aerobic nitrite reduction rate increased with FNA concentration, accompanied by an equivalent decrease in the oxygen uptake rate, suggesting under high FNA concentration conditions, electrons were channeled to nitrite reduction from oxygen reduction. In contrast, the nitrite reduction rate decreased with increased FNA concentration under anoxic conditions. Anaerobic metabolism of PAO under both anoxic and aerobic conditions was observed at high FNA concentrations. Growth of PAOs decreased sharply with FNA concentration and stopped completely at FNA concentration of 10 μg HNO_2–N/L. This study, for the first time, investigated the function of nitrite/FNA in an aerobic denitrifying phosphate removal process by evaluating electron as well as energy balances, and provides explanation for FNA inhibition mechanisms.

1. Introduction

Biological phosphorus removal can be combined with nitrogen removal in wastewater treatment plants (WWTPs). In a simultaneous nitrogen and phosphorus removal process, poly-phosphate accumulating organisms (PAOs) play an important role in denitrification and phosphate removal by using stored carbon as energy source (Meinhold et al., 1999; Zeng et al., 2003). Nitrogen removal via nitrite has a number of advantages over traditional nitrification process, including lower carbon source requirements for denitrification, lower oxygen consumption, a higher denitrification rate and less sludge production (Turk and Mavinic, 1986). These advantages are even more notable when wastewater contains high ammonium or low organic carbon contents.

However, nitrite accumulation is an unfavorable occurrence in a WWTP, as it has been reported that nitrite can slow down, or even completely stop microbial activities and reconfigure the microbial community structure (Saito et al., 2004; Pijuan et al., 2010). Indeed, nitrite has been reported to inhibit both aerobic and anoxic phosphate uptake by PAOs in enhanced biological phosphorus removal (EBPR) processes (Meinhold et al., 1999; Yoshida et al., 2006). Free nitrous acid (FNA i.e. HNO_2), the protonated species of nitrite, is likely the true inhibitor of phosphate uptake (Zhou et al., 2007). Pijuan et al. (2010) and Zhou et al. (2010) reported that aerobic and anoxic metabolism of PAOs are seriously affected by FNA.

Meinhold et al. (1999) reported that aerobic P-uptake by a PAO culture that was never exposed to nitrate or nitrite is less sensitive to FNA than anoxic P-uptake whereas Saito et al. (2004) concluded that aerobic P-uptake was more affected than anoxic P-uptake by nitrate-adapted phosphate removal culture. While Yoshida et al. (2006) also demonstrated aerobic PAO as being more sensitive to nitrite, they also pointed out that a nitrite-adapted culture would have stronger tolerance to nitrite inhibition.

It is well known that oxygen inhibits the activity of denitrifying enzymes and suppresses their synthesis, and nitrite and nitrate are less preferred electron acceptors with lower energy yields when compared with oxygen respiration (Thauer et al., 1977); however, aerobic denitrification occurs in many cases (Kucera and Dadak, 1983; Robertson and Kuenen, 1984; Wan et al., 2011) including during aerobic phosphate removal. Pijuan et al. (2010) observed nitrite reduction under aerobic conditions. They suggested that PAOs reduce nitrite for detoxification instead of energy generation. In contrast, Yoshida et al. (2006) suggested that PAOs can reduce nitrite for energy production rather than for detoxification under aerobic conditions.

Through batch tests, the present study investigates the response of an FNA-adapted PAO culture to different FNA concentrations under aerobic as well as anoxic conditions. The metabolisms of PAOs

* Corresponding author.
E-mail address: zhouyan@ntu.edu.sg (Y. Zhou).

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under these two conditions, namely phosphate uptake, nitrite reduction, poly-hydroxyalkanoate (PHA) oxidation, glycogen production, growth, and oxygen uptake rate (OUR), were measured and compared. This paper presents, for the first time, a comparative study conducted using an enriched PAO culture adapted to FNA, to examine PAOs' response to FNA inhibition under aerobic and anoxic conditions. This study further investigates and discusses the function of nitrite/FNA in the aerobic denitrifying phosphate removal process by evaluating electron as well as energy balances.

2. Methods

2.1. Sludge source

The PAO culture was withdrawn from a sequencing batch reactor (SBR) fed with synthetic wastewater containing organic sources, ammonium, orthophosphate, and a trace nutrient supplement. The reactor had been operated about 18 months before the tests. The carbon source was a mixture of acetate and propionate providing 200 mg COD/L ($COD_{Acetate}/COD_{propionate}$ is 3). Ammonium and phosphate were applied at 20 mg N—NH_4^+/L and 10 mg P—PO_4^{3-}/L, respectively. The reactor had a working volume of 4 L and was operated with a cycle time of 4 h comprising 54 min anaerobic, 60 min aerobic, and 25 min anoxic periods, followed by a second phase of anaerobic (35 min), aerobic (25 min), and anoxic (20 min) periods, and thereafter a 10 min settling and decanting period. In each cycle, 1 L synthetic wastewater was fed to the reactor in the first 8 min of the first anaerobic period and again in the first 3 min of the second anaerobic period. This resulted in a hydraulic retention time of 16 h. Sludge retention time (SRT) was maintained at 13 days. pH in the reactor was maintained in the range of 7.0–8.0 by PLC controlled acid and alkaline dosing pumps. In order to achieve simultaneous nitrification, denitrification and phosphate removal (SNDPR), the dissolved oxygen (DO) concentration during the aerobic periods was controlled at 1.0–1.5 mg/L. Nitrite accumulated to 11.6 mg NO_2^-—N/L during the aerobic phase. The SBR was displaying excellent phosphate and nitrogen removal performance when its biomass was withdrawn for the batch experiments described below. The results of a cyclic study of the parent reactor can be found in supplementary information (Fig. S1). Sludge was adapted to a FNA concentration of 0.9 HNO_2–N/L.

2.2. Batch tests

2.2.1. Sludge pretreatment

Sludge for all batch experiments, unless otherwise described, was obtained according to the following pretreatment procedures. Mixed liquor biomass was withdrawn from the SBR at the end of the cycle (anoxic phase). An anaerobic phase was allowed for 1 hour with addition of sodium acetate at 150 mg COD/L to generate the PHA pool for PAOs. Any residual COD at the end of the anaerobic phase was removed by a series of washing steps. During these washing steps, mix liquor was allowed to settle and supernatant was removed and replaced with phosphate buffer solution (PBS). Above steps were repeated three times. Washed biomass was distributed into 27 batch reactors operated in parallel, each with a working volume of 100 mL. Five mg/L of ATU (Aldrich, USA) was added to the mixed liquor to inhibit nitrification. Ammonium and phosphate were injected resulting in initial N and P concentrations of 10 mg NH_4^+—N/L and 20 mg PO_4^{3-}—P/L, respectively.

2.2.2. Sampling and reactor operation

Sampling and reactor operation protocols, unless otherwise described, were as follows. Each batch test lasted for 1 hour. During each inhibition experiment, mixed liquor samples were taken every 10 min and immediately filtered through disposable Millipore filters (0.45 μm pore size) for analysis of ammonium, nitrite and phosphate. Solids samples for the analysis of PHA and glycogen were taken every 20 min and fixed with formaldehyde (Oehmen et al., 2005). Mixed liquor samples for total suspended solids (TSS) and volatile suspended solids (VSS) measurements were withdrawn at the beginning and end of each experiment. Results obtained were normalized for biomass concentrations which were calculated using VSS subtracting PHA and glycogen content. During aerobic inhibition tests, air was supplied intermittently by an on–off controller at dissolved oxygen (DO) levels between 2–6 mg O_2/L. Anoxic condition was maintained by applying nitrogen continuously during the inhibition tests from the headspace of the batch reactor.

2.2.3. FNA inhibition tests

FNA inhibition tests were carried out with 27 FNA concentrations achieved by varying the initial nitrite concentration (0–65 mg NO_2^-—/L) and pH (6.5–8), as summarized in Table 1. The FNA concentration was calculated using $\frac{S_{N-NO_2}}{K_a^* 10^{pH}}$, with the K_a value determined using $e^{-2300/(273+T)}$ for any given temperature T (°C) (Anthonisen et al., 1976). FNA concentration of zero in the aerobic test means that the test was performed in the absence of nitrite, in which the maximum rates of each metabolic pathway was obtained. The aerobic and anoxic inhibition tests were initiated by the injection of a 60 mM nitrite stock solution into pretreated sludge. Each test lasted for 1 h. pH was controlled at the preset set-point (Table 1) using 0.1 M HCl. Reactor operation and sampling followed the protocol described in Section 2.2.2. Maximum oxygen uptake rate (OURmax) was determined using the maximum net oxygen consumption rate from each aerobic inhibition test. Detailed calculation can be found in SI.

2.2.4. Maximum P-uptake rate under anoxic condition

The maximum P-uptake rate under anoxic conditions was obtained through continuous feeding of nitrite at various loading

Table 1
Experimental conditions applied in batch tests.

Tests no. (Aerobic)	pH	NO_2^- (mg NO_2–N/L)	FNA (μg HNO_2–N/L)
1	8	0	0
2	8	11.0	0.26
3	7.5	10.6	0.79
4	7	43.5	10.3
5	6.5	61.9	46.4
6	7.5	32.2	2.4
7	7.5	42.0	3.1
8	7	27.4	6.5
9	6.5	31.6	23.7
10	6.5	40.0	30.0
11	6.5	49.0	32.8
12	6.5	51.3	36.5
13	6.5	55.1	40.1
14	6.5	67.3	50.4
Tests no. (Anoxic)			
15	8	10.7	0.25
16	7.5	10.0	0.75
17	7	39.0	9.2
18	6.5	53.3	40.0
19	7.5	30.4	2.3
20	7.5	40.7	3.1
21	7	26.3	6.2
22	6.5	29.9	22.4
23	6.5	39.0	29.2
24	6.5	46.0	32.8
25	6.5	52.1	39.0
26	6.5	55.4	41.5
27	6.5	64.9	48.6